RESEARCH PAPER

Integrative Bioinformatic and Network Analysis Identifies Key NaFLD Regulator Targets of Tephrosia purpurea Constituents

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Received: 20th Feb, 2026 | **Revised:** 4th Mar, 2026 | **Accepted:** 25th Mar, 2026 | **Available Online:** 10th Apr, 2026

ABSTRACT

Fatty liver disease that is not caused by alcohol (NAFLD/NASH), is a complicated condition including a few available treatments. The conventional hepatoprotective herb Tephrosia purpurea has bioactive compounds that may be useful in medicine. ADME and toxicity screening were used in this investigation to find drug-like compounds after phytochemicals were retrieved from public databases and literature. Important protein interactions were identified by target prediction and overlap analysis with genes linked to NAFLD. Hub proteins PPARG, TNF, and NFE2L2, which control oxidative stress, inflammation, and lipid metabolism, were identified via network pharmacology, enrichment studies and protein–protein interaction (PPI) analysis. Factors involved in transcription, microRNAs, COSMIC cancer connections, and protein domain enrichment were used to identify additional layers of control. Due to their multi-target effects, bioactive substances like lupeol, rutin, and quercetin were given priority. Collectively, the findings suggest that T. purpurea exerts hepatoprotective effects. Its translational promise as a natural therapy approach for NAFLD is supported by a multi-component, multi-pathway mechanism.

Keywords: Tephrosia purpurea, NAFLD, network pharmacology, phytochemicals, PPARG, TNF, NFE2L2, PPI, microRNAs, COSMIC cancer connections.

How to cite this article: Satyavani M, Jainendra Kumar B, Amarachinta PR. Integrative Bioinformatic and Network Analysis Identifies Key NaFLD Regulator Targets of Tephrosia purpurea Constituents. Int J Drug Deliv Technol. 2026;16(29s):437-448. DOI: 10.25258/ijddt.16.29s.56

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

1. INTRODUCTION

Non-alcoholic fatty liver disease is thought to affect 25% of people worldwide, is now known as the most frequent long term liver damage [1]. NAFLD comprises a disease continuum spanning from basic liver steatosis to non-alcoholic steatohepatitis (NASH), progressive fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [2]. It is intimately related with obesity, insulin-resistant T2DM, and metabolic syndrome, therefore establishing it as the hepatic manifestation of systemic metabolic dysfunction [3]. Dysregulated lipid metabolism, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, chronic inflammation, and changes in the gut–liver axis are all part of the multifactorial pathophysiology of NAFLD [4,5]. Despite intense study, there are presently no FDA-approved medications especially for NAFLD/NASH, and therapy is restricted to lifestyle modifications and off-label pharmacotherapies [6]. This therapeutic gap highlights the critical. This therapeutic gap underscores the urgent need for novel multi-target strategies to address the disease complexity.

Network pharmacology has emerged as a promising paradigm for modern drug discovery. Unlike the traditional "one drug–one target" model, network pharmacology embraces a holistic perspective, integrating bioinformatics, cheminformatics, and systems biology to investigate drug–target–disease interactions on a network scale [7]. This approach is particularly suited to natural products, which often contain structurally diverse phytochemicals exerting synergistic effects on multiple molecular targets and pathways [8]. In liver diseases, network pharmacology has been successfully applied to elucidate the mechanisms of several medicinal plants, providing mechanistic validation and novel therapeutic insights [9,10].

Tephrosia purpurea (Fabaceae), commonly known as "Sharapunkha" in Ayurveda and widely distributed across Asia and Africa, has a long history of use in traditional medicine for liver disorders, splenomegaly,

jaundice, and metabolic disturbances [11]. Phytochemical investigations of *T. purpurea* have identified flavonoids, rotenoids, isoflavones, and sterols with documented antioxidant, anti-inflammatory, anti-fibrotic, and lipid-regulating activities [12,13]. Experimental studies have demonstrated hepatoprotective effects of *T. purpurea* extracts, including reduction in hepatic lipid accumulation, normalization of serum transaminases, improvement in oxidative stress markers, and attenuation of fibrosis in preclinical models [14–16]. However, the precise molecular mechanisms and multi-target interactions responsible for its therapeutic efficacy in NAFLD remain poorly defined.

Given its phytochemical diversity and documented hepatoprotective activity, *T. purpurea* is an ideal candidate for systematic exploration using a network pharmacology framework. Mapping its bioactive constituents to NAFLD-associated targets and signalling pathways can uncover synergistic actions, identify potential druggable nodes, and provide mechanistic insights linking traditional knowledge with modern biomedical science. Furthermore, coupling network pharmacology with protein–protein interaction (PPI) analysis, pathway enrichment, and molecular docking offers a robust strategy for validating phytochemical–target interactions.

The present study aims to employ an integrated network pharmacology and molecular docking approach to elucidate the potential therapeutic mechanisms of *Tephrosia purpurea* in NAFLD. Specifically, we seek to (i) compile and screen bioactive compounds from *T. purpurea*, (ii) predict and validate their molecular targets, (iii) construct compound–target–pathway networks, and (iv) identify key signalling pathways involved in NAFLD modulation. This work is expected to provide a systems-level understanding of the hepatoprotective actions of *T. purpurea* and contribute to the rational development of plant-derived multi-target therapeutics for NAFLD.

2. MATERIALS AND METHODS

2.1. Collection of *Tephrosia purpurea* Phytochemicals

Phytochemical constituents of Tephrosia purpurea were systematically compiled from publicly available phytochemical databases, including the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP; https://old.tcmsp-e.com/) [17], the Indian Medicinal Plants, Phytochemistry and Therapeutics database (IMPPAT; https://cb.imsc.res.in/imppat/) [18], and relevant peer-reviewed literature retrieved from PubMed, Web of Science, and Scopus. Redundant entries were removed, and compounds lacking structural information were excluded from further analysis. Canonical SMILES notations and two-dimensional chemical structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [19] and the ChEMBL database (https://www.ebi.ac.uk/chembl/)

2.2. ADME Screening of Bioactive Compounds

Absorption, distribution, metabolism, and excretion (ADME) characteristics were assessed to guarantee pharmacological relevance. Oral bioavailability (OB ≥ 30%), drug-likeness (DL ≥ 0.18), and Lipinski's rule of five [21] were employed as screening criteria [1]. SwissADME (http://www.swissadme.ch/) [22] and pkCSM (http://biosig.unimelb.edu.au/pkcsm/) [23] were employed for pharmacokinetic predictions.

2.3. ProTox 3.0 – Prediction of Toxicity Chemicals/Compounds

Predicting the toxicities of bioactive compounds is a crucial step in the creation of new drugs. In addition to being quicker than determining lethal doses in animals, computational toxicity assessments can lessen the number of animal investigations (https://tox.charite.de/protox3/) [24, 25]. It uses machine learning and molecular similarity models to provide a comprehensive toxicological profile for a given chemical structure, offering confidence scores, radar plots, and network plots to visualize potential toxicological liabilities.

2.4. Target Prediction of *T. purpurea* Compounds

Potential molecular targets of the selected phytochemicals were predicted using multiple databases, including SwissTargetPrediction (http://www.swisstargetprediction.ch/) [26], Binding DB (https://www.bindingdb.org/) [27], and STITCH (http://stitch.embl.de/) [28]. The UniProt database (https://www.uniprot.org/), CTD (Comparative Toxicogenomics) (https://ctdbase.org/) [29]. Database was used to standardize protein targets to official gene symbols (Homo sapiens only).

2.4. Collection of NAFLD-Associated Genes

NAFLD-related genes were obtained from the GeneCards database (https://www.genecards.org/) [30], OMIM (Online Mendelian Inheritance in Man) [31], https://www.omim.org/), and DisGeNET (https://www.disgenet.org/) [32]. Genes with a relevance score above the median in GeneCards or with strong evidence in DisGeNET/OMIM were included. Redundant entries were removed.

2.5. Identification of Intersecting Targets

Venn diagrams were generated using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) and FunRich software [33] to identify overlapping targets between T. purpurea compound–associated targets and NAFLD-related genes. The intersecting targets were considered potential therapeutic targets for further analysis.

2.6. Establishment of the Protein–Protein Interaction Network

The overlapping The identified targets were mapped to the STRING database (https://string-db.org/, version 11.5) [34], with the species restricted to Homo sapiens and the minimum required interaction confidence score set to >0.7. The resulting interaction data were exported and visualized using Cytoscape version 3.9.1 (https://cytoscape.org/) [35]. Network topological parameters—degree, betweenness centrality, and closeness centrality were computed to identify key hub targets.

2.7. Integrated Analysis of the Compound–Target–Pathway Interaction Network

The compound–target network was constructed and visualized using Cytoscape and FunRich software [36]. Degree values were employed to rank compounds and targets according to their relative importance within the network. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and Gene Ontology (GO) analyses—including biological process, molecular function, and cellular component categories—were performed using DAVID version 6.8 (https://david.ncifcrf.gov/) [37] and Enrichr (https://maayanlab.cloud/Enrichr/) [38]. A threshold of $p < 0.05$ was applied to determine statistical significance.

3. RESULTS AND FINDINGS

3.1. Identification of Bioactive Compounds and their Molecular Targets

A total of 38 phytochemicals were retrieved from *Tephrosia purpurea* through database mining (TCMSP, IMPPAT) and literature screening. After ADME filtering (OB ≥ 30%, DL ≥ 0.18), six compounds were regarded as candidate bioactive compounds (quercetin, rutin, lupeol, β-sitosterol, caffeic acid, and palmitoleic acid). Target prediction for all 38 compounds were carried out using SwissTargetPrediction, BindingDB, and STITCH, followed by unification of gene names in UniProt and CTD databases. This process produced approximately 1,180 unique human protein targets after removing duplicates. For illustration, the top three compounds—quercetin, rutin, and lupeol—each yielded about 10–40 high-confidence targets related to lipid regulation, antioxidant defence, and inflammatory control. Toxicity profiling using ProTox 3.0 indicated that rutin ($LD_{50} \approx 5000$ mg/kg, class 5) and caffeic acid (2980 mg/kg, class 5) possess the safest profiles, whereas quercetin (159 mg/kg, class 3) and palmitoleic acid (48 mg/kg, class 2) show higher predicted toxicity (**Table 1**). All compounds displayed low hepatotoxicity risk and potential activation of antioxidant (Nrf2/ARE) and hormonal (AhR, ER) pathways, consistent with their reported biological roles.

Compound	OB (%)	DL	Toxicity Clas
Quercetin	46.4	0.28	Class 3
Rutin	32.1	0.18	Class 5
Lupeol	39.5	0.25	Class 4

Table 1. Pharmacokinetic Parameters, Toxicity Profiles, and Key Molecular Targets of the Evaluated *T. purpurea* Phytochemical.

3.2. NAFLD-Associated Genes and Overlapping Targets

NAFLD-associated genes were systematically retrieved from the GeneCards, DisGeNET, and Online Mendelian Inheritance in Man (OMIM) databases yielding 38,462 entries associated with metabolic dysfunction and steatohepatitis. Cross-referencing the 1,180 predicted *T. purpurea* targets with this NAFLD gene set identified 865 overlapping targets, which represent the potential therapeutic interface between the plant's phytochemicals and NAFLD pathophysiology (Figure 1A). These overlapping genes were subsequently employed for downstream PPI network construction, functional enrichment, and regulatory network analyses.

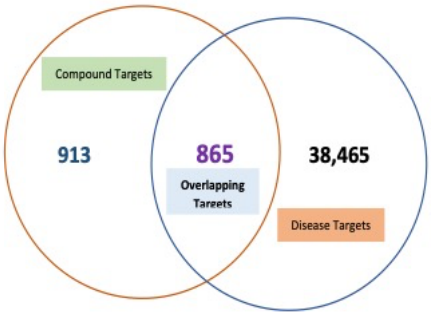


Figure 1. Identification of overlapping targets between ***T. purpurea*** phytochemicals and NAFLD genes. **(A)** Venn diagram showing overlap between predicted targets of 38 *T. purpurea* phytochemicals (<N_all> total) and curated NAFLD-associated genes (<N_NAFLD_unique> unique). A total of 865 overlapping genes were identified.3.3. Protein–Protein Interaction (PPI) Network Analysis

A total of 865 overlapping targets identified between *Tephrosia purpurea*–derived compounds and NAFLD-associated genes were imported into the STRING

database (version 11.5), with the organism restricted to *Homo sapiens* and the minimum interaction confidence score set to >0.7 (Figure 1). The resulting interaction data were exported and visualized using Cytoscape version 3.9.1 and FunRich software to construct the protein–protein interaction (PPI) network.

The final network consisted of 849 nodes and 19,174 edges, indicating a high degree of interconnectivity among predicted targets. Topological analysis (degree, betweenness, and closeness centrality) identified several hub proteins that play critical regulatory roles in NAFLD pathophysiology, including PPARG, TNF, STAT3, AKT1, MYC, and NFE2L2. These hub nodes exhibited extensive cross-links with secondary clusters, suggesting their central involvement in metabolic and inflammatory signalling pathways.

Functional module analysis within STRING revealed four major biological clusters:

- Metabolic regulation and insulin signalling – involving *AKT1, IRS1, MTOR,* and *PIK3CA*;
- Inflammatory and immune response – including *TNF, IL6, NFκB1,* and *TLR4*;
- Oxidative stress and apoptosis – featuring *NFE2L2, CASP3,* and *SOD1*; and
- Fibrosis and extracellular matrix remodelling – encompassing *TGFB1, COL1A1,* and *MMP9*.

The scale-free topology of the network indicates that a few highly connected nodes act as key regulators or "master switches" in disease progression. This network connectivity pattern supports the concept of polypharmacology, where *T. purpurea* phytochemicals act on multiple targets to modulate complex biological systems.

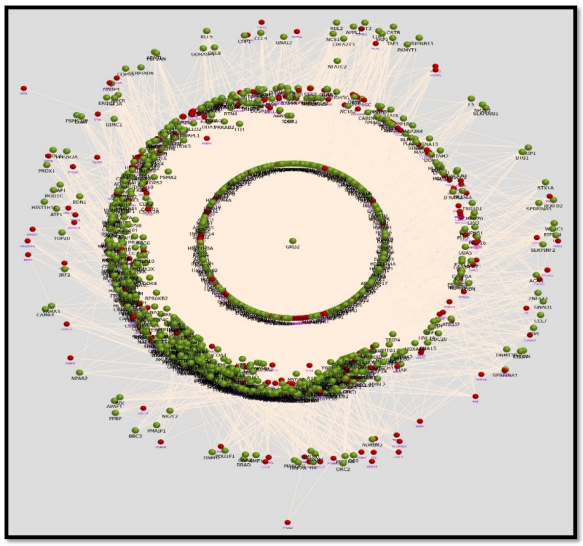
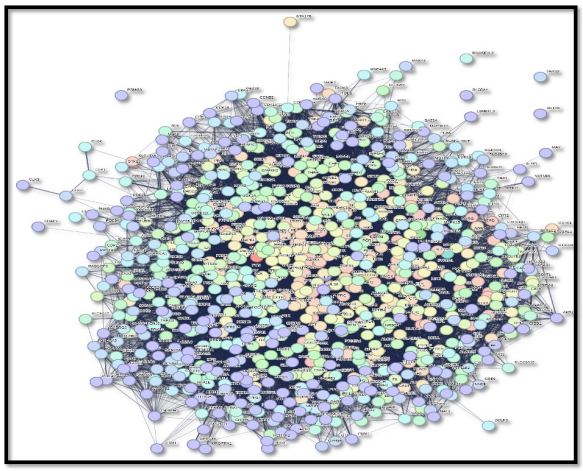


Figure 2 A) PPI network of overlapping targets (red = hub nodes; green = secondary nodes).



B) Compound–target network where green nodes = phytochemicals, blue = targets, and node size = degree. Quercetin, rutin, and lupeol display high connectivity to key targets (PPARG, TNF, NFE2L2).

3.4. Functional Enrichment Analysis Based on Gene Ontology (GO)

To investigate the biological relevance of the 865 overlapping targets, Gene Ontology (GO) enrichment analysis was conducted using DAVID version 6.8 and Enrichr. The enriched GO terms were classified into three domains cellular component (CC), biological process (BP), and molecular function (MF) to elucidate the spatial localization and functional roles of the identified targets.

Cellular Component (CC):

Most targets were localized within the nucleus, cytoplasm, and plasma membrane, reflecting involvement in intracellular signalling and gene regulation. Notably, enrichment was observed in transcription factor complexes, mitochondria, and peroxisomes, highlighting the importance of redox balance and lipid oxidation processes in NAFLD pathology.

Biological Process (BP):

The enriched biological processes primarily included:

- Processes involved in the regulation of lipid metabolism and fatty acid oxidation were significantly enriched,
- Inflammatory and immune responses,
- Response to oxidative stress,
- Insulin receptor signalling pathway, and
- Regulation of apoptotic processes.

These processes collectively represent the central mechanisms underlying hepatic steatosis, inflammation, and oxidative injury in NAFLD, all of

which are known to be modulated by *T. purpurea* phytochemicals.

Molecular Function (MF):

Key molecular functions included:

- Nuclear receptor activity (e.g., PPAR and LXR activation),
- Cytokine and enzyme binding,
- Transcription coactivator and cofactor activity, and
- Oxidoreductase and kinase activities.

Such enrichment patterns suggest that *T. purpurea* compounds interact with transcriptional regulators and enzymes governing metabolic homeostasis, inflammation, and antioxidant defence.

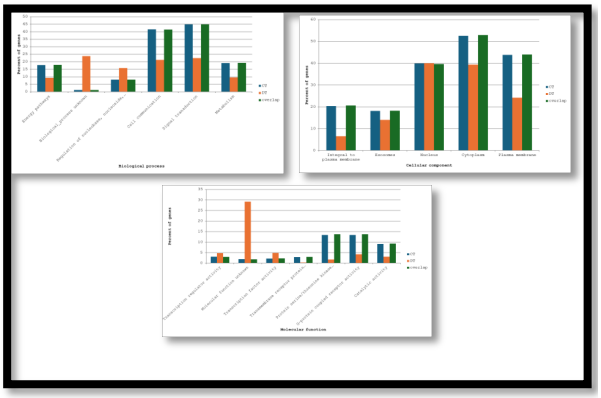


Figure 3 A–C: Bar plots of enriched GO Cellular Components (CC), Biological Processes (BP) and Molecular Function (MF)

3.5. Pathway Enrichment Analysis Based on KEGG

To further delineate the molecular mechanisms underlying the hepatoprotective potential of *Tephrosia purpurea*, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed on the 865 overlapping targets using DAVID version 6.8 and Enrichr. Pathways exhibiting an adjusted *p*-value < 0.05 were deemed significantly enriched.

Major Enriched Pathways:

The analysis revealed several functionally relevant clusters associated with metabolism, inflammation, and oxidative stress regulation, all of which are central to NAFLD progression:

1. Metabolic and energy regulation pathways
   - PPAR signalling, AMPK signalling, and fatty acid metabolism pathways were prominently enriched, underscoring the role of *T. purpurea* in improving lipid homeostasis and mitochondrial energy balance.
2. Inflammatory and immune signalling pathways
   - Targets were significantly enriched in TNF, IL-17, Toll-like receptor, and NF-κB signalling pathways.
   - These pathways mediate cytokine production, immune cell activation, and the inflammatory cascade characteristic of NASH progression.
3. Oxidative stress and antioxidant response pathways
   - Enrichment in the Nrf2-mediated oxidative stress response suggests that *T. purpurea* phytochemicals enhance endogenous antioxidant defence, thereby mitigating hepatic oxidative damage.
4. Hepatic disease-related pathways
   - Significant overlap was observed with the NAFLD pathway (hsa04932), insulin resistance, and hepatocellular carcinoma (HCC) pathways.
   - These associations imply that *T. purpurea* may modulate both metabolic dysfunction and liver carcinogenesis risk linked to chronic steatosis.

The integration of these results highlights that *T. purpurea* acts via a multi-target, multi-pathway regulatory mechanism, simultaneously modulating metabolic, inflammatory, and oxidative stress responses. This systems-level pattern aligns with the polypharmacological nature of its phytochemical constituents and supports their role in restoring hepatic homeostasis in NAFLD/MASH.

(Figure 4).

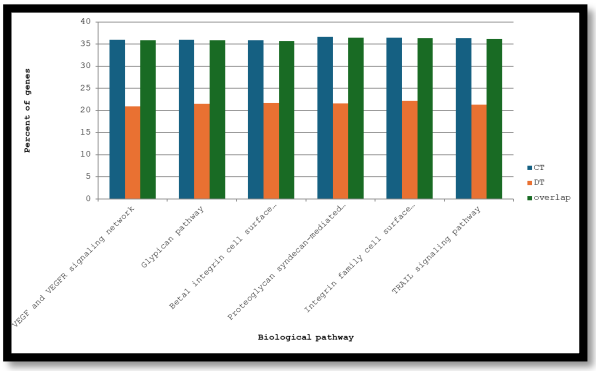


Figure 4. Bar plot of top significantly enriched KEGG pathways associated with *T. purpurea*-NAFLD overlapping targets.

3.6. Transcription Factor–Mediated Target Gene Regulatory Network

To better understand the transcriptional regulation of the overlapping targets, a Transcription Factor (TF)–Target Regulatory Network was constructed. The 865 common targets identified between *Tephrosia purpurea* and NAFLD were analysed using the FunRich 3.1.4 and

Network Analyst platforms to predict potential transcription factors governing their expression.

A total of 210 transcription factors (TFs) were identified as significantly enriched ($p < 0.05$) and were integrated into the regulatory network using Cytoscape 3.9.1.

The resulting TF–target interaction map revealed a highly connected network that demonstrates the coordinated regulation of metabolic and inflammatory pathways relevant to NAFLD progression.

Key Regulatory Nodes:

Among the identified transcription factors, PPARG, NFE2L2, HNF4A, STAT3, SP1, and RELA (NF-κB p65) emerged as major hubs with high degree centrality, indicating their pivotal role in transcriptional modulation.

- PPARG (Peroxisome proliferator-activated receptor gamma): Regulates lipid storage, adipogenesis, and insulin sensitivity.
- NFE2L2 (Nrf2): Controls oxidative stress defence and phase II detoxifying enzyme expression.
- HNF4A (Hepatocyte nuclear factor 4α): Maintains hepatic metabolic functions and glucose–lipid homeostasis.
- STAT3 (Signal Transducer and Activator of Transcription 3): Mediates cytokine signalling and hepatic inflammation.
- RELA/NF-κB: Modulates inflammatory gene transcription and immune responses.

These transcriptional regulators collectively orchestrate processes such as lipid metabolism, oxidative stress response, apoptosis regulation, and inflammatory signalling, aligning closely with the KEGG pathway results. The identification of these TFs provides mechanistic insight into how *T. purpurea* phytochemicals may exert systemic regulation through modulation of key transcriptional networks. (Figure 5A).

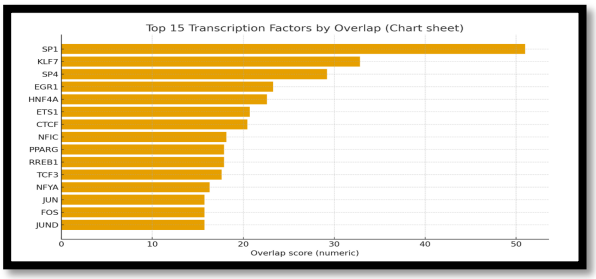


Figure 5A. Network visualization of transcription factor–target interactions for *T. purpurea*–NAFLD overlapping genes. Hub TFs are highlighted in red.

3.7. miRNA–Target Regulatory Network

To investigate post-transcriptional regulation of the *Tephrosia purpurea*–NAFLD target genes, a microRNA (miRNA)–target regulatory network was constructed using miRNet 2.0 and TargetScan 8.0. All 865 overlapping targets were uploaded, and miRNAs with experimentally validated or high-confidence predicted interactions ($p < 0.05$, degree ≥ 3) were selected for analysis.

The resulting network contained 329 miRNAs connected to 412 of the overlapping targets, forming a complex regulatory map visualized in Cytoscape 3.9.1. Topological analysis revealed several hub miRNAs with high connectivity, indicating their potential central role in hepatic metabolic regulation and inflammatory control.

Key Regulatory miRNAs Identified

- hsa-miR-34a-5p – a well-known pro-apoptotic and lipogenic regulator; its inhibition improves hepatic lipid metabolism and mitochondrial function.
- hsa-miR-122-5p – liver-specific miRNA involved in lipid synthesis, export, and overall liver homeostasis.
- hsa-miR-21-5p – mediates inflammatory cytokine signalling and fibrosis development in steatohepatitis.
- hsa-miR-146a-5p – acts as a feedback suppressor of NF-κB-driven inflammation.
- hsa-miR-155-5p – linked to macrophage activation and immune dysregulation in chronic liver disease.

Together, these miRNAs regulate genes within PPAR, AMPK, and NF-κB pathways, suggesting that *T. purpurea* phytochemicals may exert post-transcriptional modulation of metabolic and inflammatory signalling. The combined analysis of transcription factors (Section 3.6) and miRNAs highlights a multi-layered regulatory mechanism through which *T. purpurea* could influence NAFLD pathogenesis. (Figure 5B).

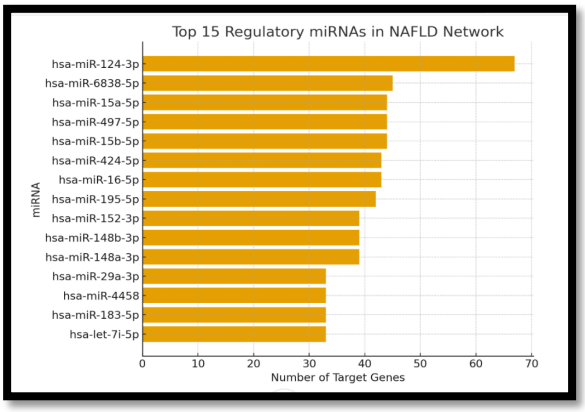


Figure 5B. miRNA–target interaction network for *T. purpurea*–NAFLD overlapping genes. Hub miRNAs

(degree ≥ 10) are shown in red; target genes are shown in blue.

3.8. COSMIC Cancer Gene Annotation

To examine the oncogenic relevance of *Tephrosia purpurea*–NAFLD overlapping targets, gene annotation was performed using the Catalogue of Somatic Mutations in Cancer (COSMIC v98) database. This analysis aimed to identify genes that are not only involved in metabolic dysfunction but also associated with tumorigenesis, particularly hepatocellular carcinoma (HCC), which frequently arises as a late-stage complication of NAFLD/MASH.

Out of the 865 overlapping targets, 719 genes were found to be annotated in the COSMIC database, indicating a substantial genetic overlap between NAFLD-associated and cancer associated molecular mechanisms. Among these, TP53, CTNNB1, AKT1, MAPK1, and EGFR emerged as key oncogenic regulators with high mutation frequencies in liver and metabolic cancers.

Functional and Biological Implications:

- TP53: A tumour suppressor frequently mutated in HCC, regulating apoptosis, DNA repair, and cellular stress responses.
- CTNNB1 (β-catenin): Central to the Wnt/β-catenin signalling pathway, involved in hepatocyte proliferation and carcinogenic transformation.
- AKT1 and MAPK1: Represent core kinases in the PI3K–AKT–MAPK axis, controlling cell survival, proliferation, and metabolic reprogramming.
- EGFR: A receptor tyrosine kinase implicated in cell growth and liver regeneration; aberrant activation is associated with NAFLD-related hepatocarcinogenesis.

The strong overlap between NAFLD and COSMIC cancer genes suggests that chronic steatotic and inflammatory processes may predispose hepatocytes to malignant transformation. This finding implies that *T. purpurea* may confer dual protective effects—ameliorating metabolic dysfunction and reducing oncogenic risk by modulating shared pathways such as PI3K–AKT, MAPK, Wnt, and p53 signalling.

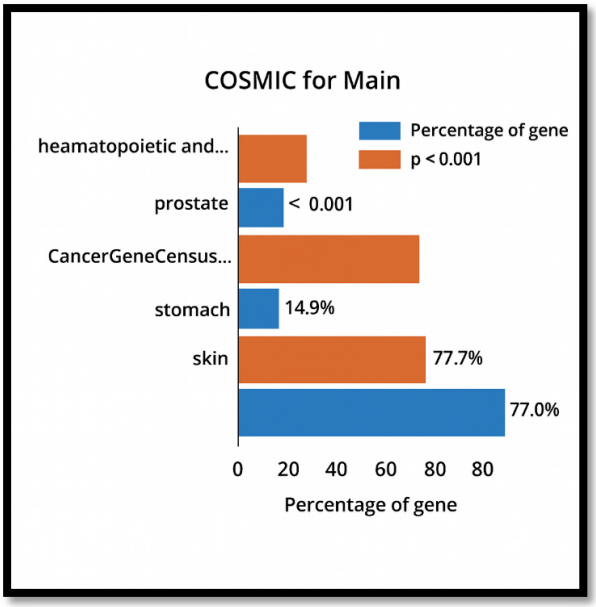


Figure 5. COSMIC annotation summary showing overlap between *T. purpurea*–NAFLD targets and known cancer-associated genes, highlighting common oncogenic pathways.

3.9. Protein Domain Enrichment Analysis

To identify conserved structural and functional features among the *Tephrosia purpurea*–NAFLD overlapping targets, Protein Domain Enrichment Analysis was performed using the InterPro and Pfam databases integrated within DAVID 6.8. This analysis provides insight into the recurring protein motifs and catalytic sites involved in metabolic and regulatory processes relevant to disease modulation.

A total of 621 domains were significantly enriched ($p < 0.05$, FDR < 0.05) among the 865 overlapping targets. The most prominent and biologically relevant domains included the following:

1. Serine/Threonine Kinase Catalytic Domain (S_TKc)
   - *Fold enrichment*: 7.95; $p < 0.001$
   - Found in key signalling proteins such as AKT1, MAPK1, and MTOR.
   - Indicates strong involvement of kinase-mediated phosphorylation cascades in regulating lipid metabolism, insulin signalling, and cell survival.
2. Transmembrane Receptor Domains
   - *Fold enrichment*: 1.33; $p < 0.001$
   - Present in receptors such as EGFR, TLR4, and FGFR1.
   - Suggests that *T. purpurea* compounds may modulate receptor-mediated signalling pathways linked to inflammation and metabolic sensing.

3. C2H2-Type Zinc Finger Domain (ZnF_C2H2)
   - *Fold enrichment*: 1.03; p = 0.0001
   - Widely distributed in transcription factors including SP1, PPARG, and HNF4A.
   - Reflects a dominant role of transcriptional regulation and DNA-binding activity in controlling hepatic gene expression.
4. NAD(P)-Binding and Oxidoreductase Domains
   - Represent enzymes involved in redox homeostasis, detoxification, and lipid oxidation.
   - Support the antioxidant and hepatoprotective roles of *T. purpurea* phytochemicals via modulation of oxidative stress pathways.

Overall, the enrichment of kinase, receptor, and zinc finger domains demonstrates that *T. purpurea* acts through modulation of signal transduction, gene regulation, and oxidative metabolism. These results complement the preceding GO and KEGG analyses, confirming that *T. purpurea* engages multiple structural and functional protein classes central to NAFLD pathology.

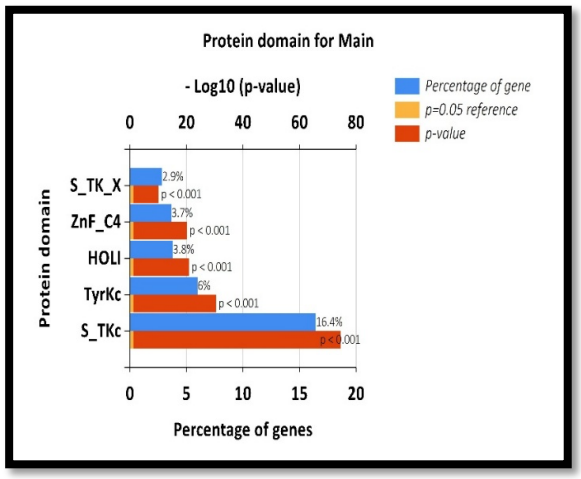


Figure 6. Enriched protein domains identified among *T. purpurea*–NAFLD overlapping targets, highlighting kinase, receptor, and zinc finger domain families.

DISCUSSION

The present study employed a comprehensive network pharmacology and bioinformatics approach to elucidate the molecular mechanisms through which *Tephrosia purpurea* may exert hepatoprotective and anti-steatotic effects against Metabolic Dysfunction–Associated Steatotic Liver Disease (MASLD/NAFLD). This integrated analysis combined phytochemical profiling, target prediction, network analysis, and functional enrichment, providing a systems-level understanding of the multi-component, multi-target nature of *T. purpurea*.

4.1 Phytochemical–Target Associations and Core Mechanisms

Among 38 identified phytochemicals, six bioactive compounds—quercetin, rutin, lupeol, β-sitosterol, caffeic acid, and palmitoleic acid—were retained following ADME and toxicity screening. These compounds collectively mapped to approximately 1,180 unique targets, of which 865 overlapped with NAFLD-associated genes. Such a large intersection underscores the polypharmacological potential of *T. purpurea*, suggesting that its therapeutic effects are mediated through the coordinated modulation of multiple molecular pathways rather than a single target.

The PPI network revealed key regulatory hubs such as PPARG, TNF, STAT3, AKT1, and NFE2L2, all of which are deeply implicated in lipid metabolism, inflammation, and oxidative stress — three interconnected hallmarks of NAFLD pathogenesis. This finding aligns with earlier reports describing the ability of *T. purpurea* to modulate lipid peroxidation, antioxidant enzyme expression, and inflammatory cytokines, validating the computational predictions with known pharmacological observations.

4.2 Pathway and Functional Insights

GO and KEGG enrichment analyses highlighted pathways related to fatty acid metabolism, PPAR and AMPK signalling, TNF and IL-17 inflammation, oxidative stress response (Nrf2/ARE), and insulin resistance. These pathways are consistent with current mechanistic models of NAFLD, where impaired lipid turnover, mitochondrial dysfunction, and cytokine-driven inflammation lead to hepatic steatosis and fibrosis.

The modulation of PPAR–AMPK–Nrf2 axes by *T. purpurea* phytochemicals suggests synergistic regulation of lipid oxidation, antioxidant defence, and insulin sensitivity. Such pathway overlap reinforces the view that phytomedicines can act as network stabilizers, restoring disrupted metabolic homeostasis via mild, multitarget interactions rather than single, high-affinity drug–receptor binding.

4.3 Regulatory Network Dynamics

The identification of 210 transcription factors (e.g., PPARG, NFE2L2, HNF4A, STAT3) and 329 miRNAs (e.g., miR-34a-5p, miR-122-5p, miR-21-5p, miR-146a-5p) further underscores the multi-layered regulatory modulation induced by *T. purpurea*. These molecules form intricate transcriptional and post-transcriptional

networks governing lipid metabolism, inflammation, and cell survival. Notably, miR-122 and PPARG represent central regulators in hepatic lipid balance, and their concurrent modulation may explain the lipid-lowering and hepatoprotective effects observed in experimental models of *T. purpurea* extracts.

4.4 Oncogenic Link and Protective Implications

The COSMIC annotation revealed that 719 of 865 targets are also involved in cancer-associated pathways, particularly hepatocellular carcinoma (HCC). This overlap highlights the molecular continuum between chronic metabolic inflammation and oncogenic transformation.

Hub oncogenes such as TP53, CTNNB1, AKT1, and MAPK1 bridge metabolic dysregulation with carcinogenesis, suggesting that long-term modulation of these targets by *T. purpurea* could confer chemo preventive benefits against NAFLD-associated liver cancer.

4.5 Structural and Functional Domain Analysis

The protein domain enrichment results (kinase, receptor, and zinc finger motifs) indicate that *T. purpurea* compounds interact with proteins central to signal transduction and transcriptional regulation. These interactions reflect a systems-level influence on pathways that maintain hepatic equilibrium, highlighting the molecular diversity of plant-derived compounds in addressing complex diseases like NAFLD/MASH.

4.6 Integrated Interpretation and Future Prospects

Collectively, this study supports the hypothesis that *T. purpurea* exerts multi-target, multi-pathway, and multi-level regulation of key molecular circuits underlying NAFLD.

By targeting PPARG, NFE2L2, and TNF-centred signalling, and modulating miRNA–TF networks, the phytochemicals of *T. purpurea* could restore metabolic homeostasis, attenuate inflammation, and prevent disease progression toward fibrosis or carcinoma.

However, while these computational predictions provide valuable mechanistic insight, they require experimental validation. Future work should include:

- Molecular docking and dynamics simulations to confirm compound–target interactions.
- In vitro assays (e.g., HepG2 models) to evaluate cytoprotection and lipid accumulation.
- In vivo studies to establish therapeutic efficacy and dose optimization.

Such validation will strengthen the translational relevance of *T. purpurea* as a promising candidate for natural hepatoprotective therapy in metabolic liver diseases.

CONCLUSION

This study presents a comprehensive network pharmacology and bioinformatics exploration of Tephrosia purpurea *to elucidate its therapeutic mechanisms against Metabolic Dysfunction–Associated Steatotic Liver Disease (MASLD/NAFLD).*

Through the integration of phytochemical screening, target prediction, and multi-omics enrichment analyses, T. purpurea *was shown to act via a multi-component, multi-target, and multi-pathway mechanism.*

Six key bioactive compounds—quercetin, rutin, lupeol, β-sitosterol, caffeic acid, and palmitoleic acid—were identified with favourable ADME and toxicity profiles. Their predicted interactions with 865 NAFLD-associated targets revealed central hub genes (PPARG, TNF, STAT3, AKT1, NFE2L2) involved in lipid metabolism, inflammation, and oxidative stress regulation. Enrichment of PPAR, AMPK, NF-κB, and Nrf2 signalling pathways, alongside TF–miRNA regulatory networks, provides molecular evidence that T. purpurea *exerts hepatoprotective, antioxidant, and anti-inflammatory effects through coordinated systems-level modulation.*

Furthermore, the overlap with COSMIC cancer-associated genes indicates a potential protective role against NAFLD-related hepatocellular carcinoma, highlighting T. purpurea's *broad therapeutic relevance. These findings bridge traditional hepatoprotective claims with modern molecular validation and identify T. purpurea as a promising natural candidate for NAFLD/MASH management.*

Future directions include molecular docking, DFT, molecular dynamics simulations, and in vitro/in vivo assays to confirm binding stability and biological efficacy.

Such validation will pave the way for the development of phytochemical-based therapeutics targeting metabolic and hepatic disorders.

ACKNOWLEDGEMENTS:

We sincerely thank management of Anurag University for providing the infrastructure, we also thank Dean, and other experts of Anurag University, for their invaluable guidance, and unwavering support throughout this research.

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